ARTIFICIAL CULTURES OF ASCOBOLUS AND ALEURIA

B. O. Dodge

(With Plates 72 and 73, Containing 11 Figures)

Methods by which any considerable number of species of discomycetes can be successfully cultivated on artificial media have not as yet been worked out, and any additional information along this line may be of interest because of the value of such methods in connection with the study of the reproductive processes and the identification of species.

The writer has recently found a species of *Ascobolus* which seems to be quite different from any species described, both as to the characters recognized by the ordinary methods and those which can be brought out satisfactorily only by cultures from the germinated ascospores. The species has been under observation for about three months and has been grown on the natural substratum by transferring pieces of dung bearing young fruits, and has also been brought to maturity on agar media, the cultures having been started by germinating the ascospores by the methods already described (Bull. Torrey Club **39**: 139–197). A more detailed account of the methods of reproduction, determined by a study of the fungus in the artificial cultures, will be given later, such reproductive features being noted at this time as can be observed without resorting to artificial cultures.

Ascobolus magnificus sp. nov.

Ascocarps scattered or closely crowded together, sessile, at first globose, closed, white or whitish, opening by a pore, the smooth white margin inrolled, becoming deeply cup-shaped, the lower portion of the exterior appearing pruinose from the projecting tips of thin-walled, hair-like hyphal branches which later become discolored and brownish, finally expanding, exposing the pale greenish-yellow surface of the hymenium, .5–2.7 cm. in diameter; asci cylindric-clavate, 200–300 × 18–25 μ, I +, 8-spored;

paraphyses linear, slightly enlarged above, septate, frequently with greenish, granular contents, 5–7 μ; spores ellipsoid, at first hyaline, then pale-lilac, finally rose-purple or violet, smooth, marked on one side by a line extending from end to end or obliquely across the surface, irregularly distichous at maturity, 20–25 × 12–14 μ, usually germinating at only one germ-pore; archicarp consisting of a stalk of 3–4 thick cells, a somewhat spherical ascogenous cell 35–45 μ in diameter, and a trichogyne with 7–10 cells, the outer cells coiling sharply inward at the tip, the complete archicarp coiled in one plane.

On horse dung in damp chamber cultures, New York City, April, 1912; type specimens deposited in the herbarium of the New York Botanical Garden.

The principal characters which distinguish this species are the large size of the plants, the beautiful white margin, the line extending across the surface of the spore, a single germ-tube, and the large archicarp in a flat coil. *Ascobolus applanatus* (Rabh. & Gonn.) Rehm, which Rehm (Disc. p. 1131) considers a doubtful species, is said to be 2 cm. in diameter; as to the other characters noted, it does not resemble this species. *A. major* B. & C., and *A. sarawacensis* Ces. are large species with smooth spores. *A. latus* Penz. & Sacc. and *A. laevisporus* Speg. are evidently more nearly related to *A. magnificus* but differ in the spore markings.

The line along the surface of the spore is visible before the spore becomes colored, and is not in the nature of a crack in the epispore, although a crack frequently develops along this line when the ejected spore is allowed to dry out; under such conditions numerous other cracks are formed in all directions, giving the spore a reticulated appearance.

The pruinosity of the exterior of the ascocarp would probabiy not be noticeable were the fungus grown in the open. Even in damp chambers, when specimens (Pl. 72, f. 4) become fully expanded and flattened out on the substratum (Pl. 73, lower figure), this pruinosity is no longer evident.

The very hard and brittle character of the thick flesh of the hypothecium is indicated by the way in which the ascocarps crack while expanding. (Plate 73, upper figure.)

The asci do not project prominently above the surface of the

hymenium, and after the whole surface has become deeply colored purple with ripe spores, on lifting the cover of the damp chamber the spores will be shot off in a cloud, just as is commonly the case with many of the large fleshy discomycetes.

The damp chamber cultures in which this fungus made its appearance had been kept about two weeks in a Wardian case, where they were exposed to the direct sunlight during part of the day. The substratum had been heavily watered while yet fresh so that at this time the mass was in a very putrid condition. The excessively high temperatures prevailing in this room and the condition of the substratum may perhaps account for the production of mature fruit bodies in which no colored spores were formed. In these cases all ejected spores were perfectly hyaline. Many of these spores had already germinated within the asci, and they also germinated readily in agar media without special treatment. When, however, the cultures were removed to a cooler room, colored spores were formed. As it was difficult to obtain uncontaminated cultures on agar by using the uncolored spores, several plates were inoculated with the colored spores and heated for thirty minutes in an oven, the final temperature of the oven being about 70° C. Spores in all the plates germinated. The ascocarps do not mature well on the agar media and it has been more satisfactory to transplant pieces of agar containing the mycelium or young fruit bodies to the dung where the supply of nutrient is less limited. Plate 73, upper figure, shows a culture obtained in this manner.

While fully 50 per cent. of the spores germinated in the earlier experiments, in the case of spores gathered about ten days later not over 1 per cent. could be made to grow by the heating process, and none germinated without heating.

Pure cultures of the species have not been obtained on account of the presence of a fungus which is parasitic on the mycelium of the *Ascobolus*. This parasite forms large numbers of fruit bodies, consisting when young of a central cell enclosed by protecting hyphae. It has been possible to trace a direct connection between the mycelium of the parasite bearing these fruit bodies and the mycelium of the host bearing its characteristic archicarp. As no spores of any description have been discovered,

further investigation will be necessary before the identity of the parasite can be determined. Portions of the mycelia of the host and of the parasite are shown in Plate 72, figs. 7 and 8.

ALEURIA UMBRINA Boud.

This fungus grows on burned places during the early part of the season in the vicinity of New York City. The species has been identified by Dr. F. J. Seaver. The outer surface is coarsely warted, especially in young specimens, where the stipe imbedded in the earth is also seen to be well developed. *Plicaria echinospora* (Karst.) Rehm, has been recorded as growing on burned places and the two species are apparently closely related.

Pure cultures of this species may be obtained easily by growing the spores on an agar medium made up with an extract of heated soil. When the spores are heated to 70°–80° C. for fifteen minutes, as described under "*Ascobolus carbonarius*" (Bull. Torrey Club 39: 139–197), germination is above 90 per cent. A large germ-tube is first formed and is usually followed later by a smaller one at the opposite end of the spore. (Pl. 72, f. 9.)

COLUMBIA UNIVERSITY,
NEW YORK CITY.

EXPLANATION OF PLATE LXXII

Ascobolus magnificus Dodge

Fig. 1. (*a*) Ejected spores before drying. (*b*) After drying out the epispore is cracked in all directions. × 525. The width of the cracks is slightly exaggerated in the drawing.

Fig. 2. (*a*) Ungerminated spores. (*b*) Germinated spore. × 525.

Fig. 3. (*a*) Germinated spores. (*b*) A large spore much swollen. × 525.

Fig. 4. Section through an ascocarp showing hymenial layer and the tips of secondary mycelial hyphae (?) appearing as hairs on the exterior.

Fig. 5. Asci and paraphyses. (*a*) × 100; (*b*) × 300.

Fig. 6. Archicarp as it appears in youngest fruit bodies that can be seen with a hand lens. (*s*) The stalk; (*a*) ascogenous cell; (*t*) trichogyne. The size of the ascocarps at this time is indicated by the border line. × 150.

Figs. 7, 8. (*a*) Mycelium of a fungus parasitic on the mycelium. (*b*) Mycelium of the *Ascobolus*. × 525.

Aleuria umbrina Boud.

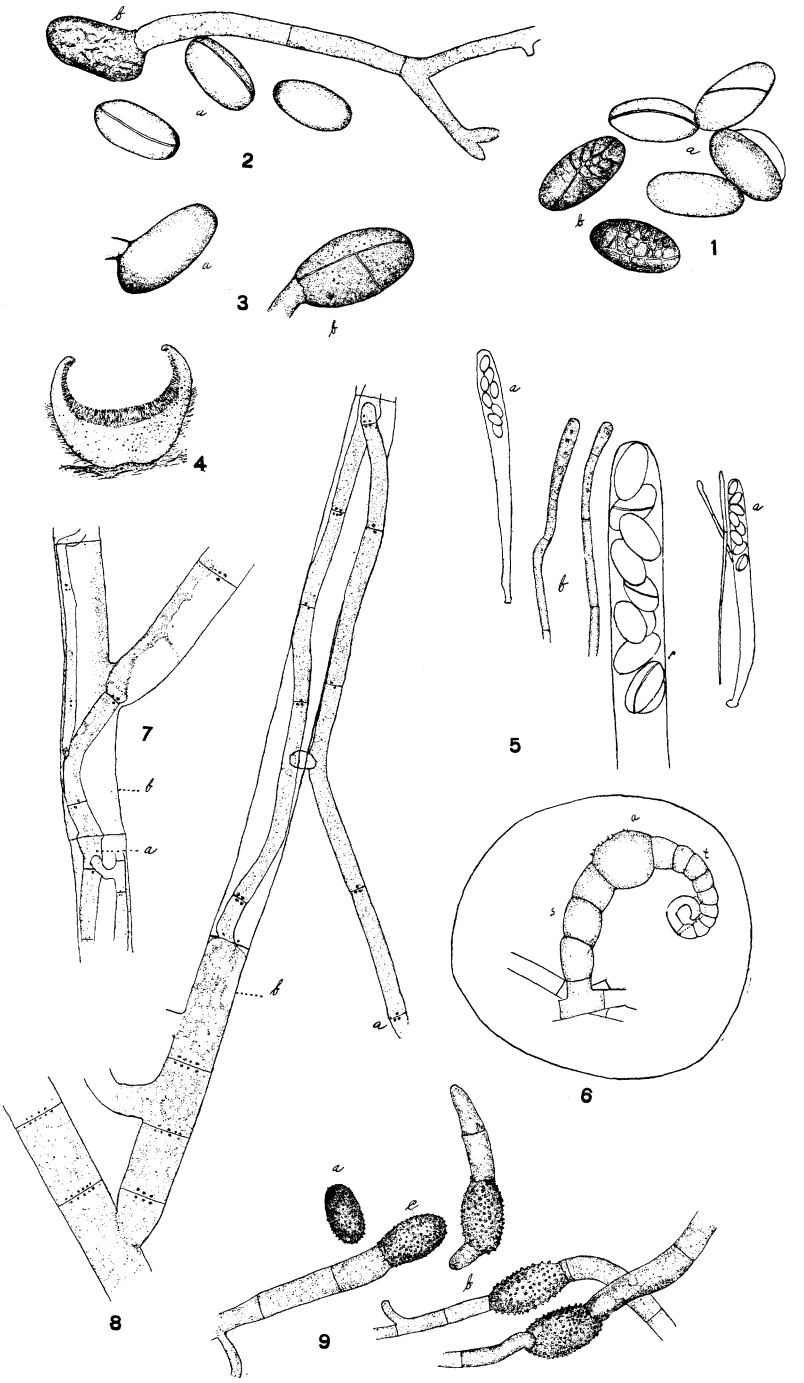
Fig. 9. (*a*) Ungerminated spore. (*b*) Germinated spores. (*c*) A spore with only one germ-tube. × 525.

EXPLANATION OF PLATE LXXIII

Ascobolus magnificus Dodge

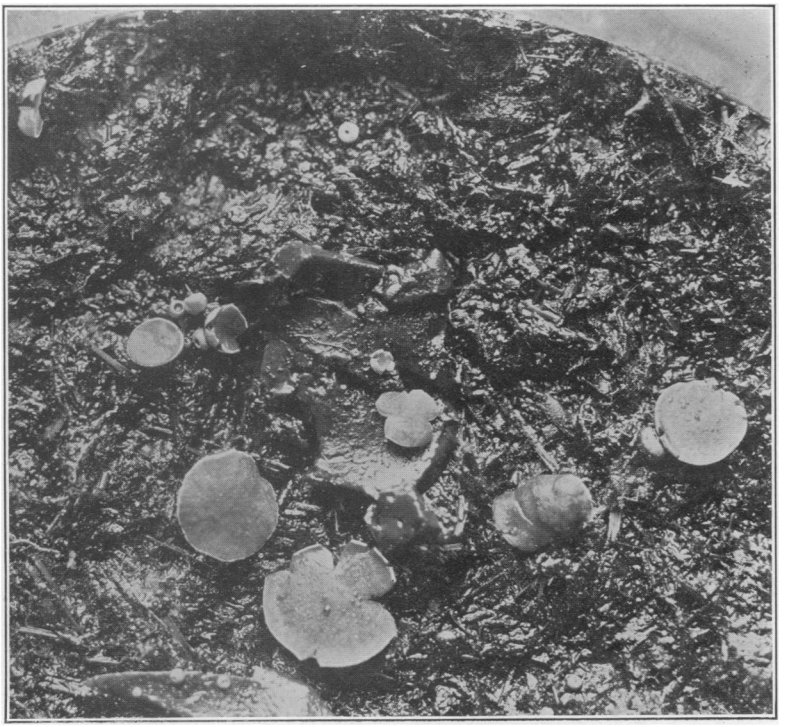
Two rather large ascocarps are shown natural size in the lower figure, the character of the white margin well brought out. At the extreme left may be seen two small fruit bodies. Seventeen ascocarps were later developed at this point, forming a compact mass of fruit bodies, each being about 1 cm. in diameter.

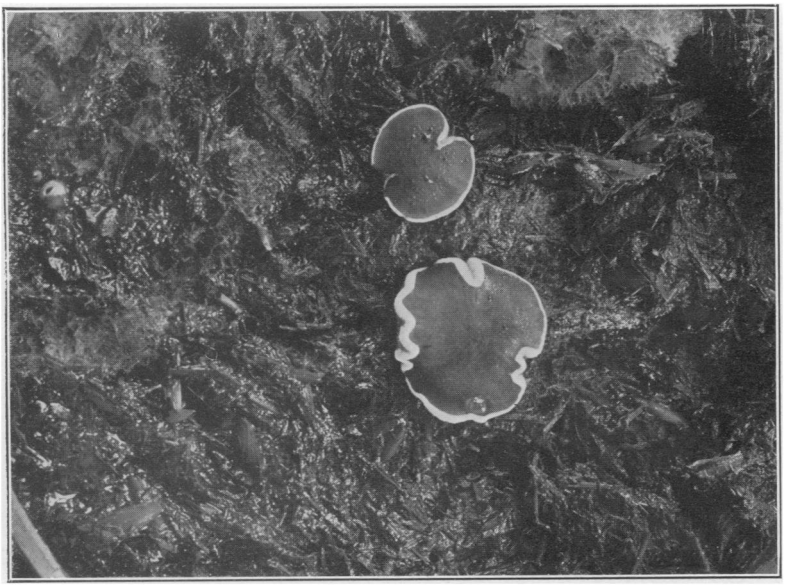
In the upper figure, are a number of young ascocarps showing the pore at the time of opening; the mature ascocarps are about the average size.



1–8. ASCOBOLUS MAGNIFICUS DODGE

9. ALEURIA UMBRINA BOUD.





ASCOBOLUS MAGNIFICUS DODGE